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CONFERENCE NEWS

Cold Spring Harbor Asia/International Society for Stem Cell Research Conference; Cellular Programs and Reprogramming (October 24–28, 2011)

The Cold Spring Harbor annual meeting, "Cellular Programs and Reprogramming", was held at the Dushu Lake Conference Center, Suzhou, China. The meeting started with welcoming remarks by Ronald D. McKay (National Institute of Health, USA) followed by the opening keynote address delivered by Elaine Fuchs (Rockefeller University, USA; Cell Stem Cell 10, 63-75, 2012; Nat. Rev. Mol. Cell. Biol. 13, 103-114, 2012; Cell 144, 341-352; Cell 92-105, 2011), who summarized her research on skin stem cells and wound repair and cancer. Using H2B-GFP pulse, chase and trace of imaging, she described the determination of how extrinsic signaling to stem cells sets off a cascade of changes in transcription that governs the activation, polarization, and migration of stem cells during tissue development, homeostasis, hair cycling, and wound repair. She demonstrated the cross-talk between the Wnt, BMP, and TGFβ signaling pathways of stem cells and niches during wound repair, quiescence, and the arrest of the generation of new tissue. In nucleus of stem cells, the expression of H3K4me3, H3K79me2, H3K27me3, and polycomb proteins is critical for the determination of stem cell-niche interactions.

Human stem cells

Fred Gage (The Salk Institute for Biological Studies, USA; Nature 473, 221-225, 2011) described neural-subtype-specific differentiation using human ESC and iPSC technology, to generate hippocampal granule neurons and their related disease, schizophrenia. The prospero homeobox protein 1 (Prox1) provides a useful *in vitro* model for studying neurogenesis in development and disease, as well as a human-based platform for drug screening for human system. Gong Chen (Pennsylvania State University, USA) established the protocol of human iPSCs that is used to differentiate functional neuron-like astroglial cells. Action potentials and synaptic response were detected within glial support. Gabriella Ficz and Wolf Reik (The Babraham Institute, Cambridge, UK; Nature 473, 398-402, 2011) reported the roles of hydroxymethylation and TET proteins (conversion of 5mC to 5hmC) in reprogramming and pluripotency in early embryos and in stem cells. 5hmC is enriched in the euchromatin of mouse ESCs via the action of TET1. Linzhao Chen (Johns Hopkins University, USA) described the reprogramming method based on the ENBA1/ori P episomal vector for cord blood and adult PBLs. Meng Li (Imperial College London, UK) and Joan Li (University of Queensland, Australia) reported the productions of functional dopamine neurons and the maturation of ESCs and mesenchymal stem cells (MSCs) into neonatal kidneys. Finally, Richard Yang (MIT, USA; Cell 144, 940-954, 2011) summarized his studies of the transcriptional networks involved in cell-fate determination.

Control of pluripotency

Marius Wernig (Stanford University, USA; Nature 476, 220-223, 2011; Nat. Biotechnol. 29, 892-907, 2011) reported the direct conversion of fibroblasts to neuronal cells *in vitro* (termed induced neuronal cells [iNCs]). Previously, this author and coworkers reported that the bHLH transcription factors Ascl1 and Mytl1, and Pou-domain transcription factor (Brn2/4 = Pou3f2) are critical for the induction of iNCs and generated functional iNCs using FoxG1 and Sox2 for astrocytic differentiation. The addition of Brn 2 to iNCs induced the tripotent iNSCs (astrocytes, neurons, and oligodendrocytes). Wenyu Zhou and H. Ruohola-Baker (University of Washington, USA) described the differential roles of hypoxia-inducible factors (HIFs) in pluripotency. Low oxygen tension (or hypoxia) facilitates stem cell proliferation and maintenance. HIF1α is beneficial for the induction of iPSCs and HIF2α dominantly represses reprogramming via the TNF-related apoptosis-inducing ligand (TNFSF10, TRAIL). Shuibing Chen (Weill Cornell Medical College, USA) and Jennifer Nichols (University of Cambridge, UK) reported on chemicals that maintain of hESCs and naïve pluripotency in early mammalian embryos,

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respectively. Yi Zhang (The University of North Carolina, USA; Science 333, 1300-1303, 2011; Science 334, 194, 2011) described the critical role of the TET1-3 enzymes in the generation of 5mC, 5hmC, 5-formylcytosine (5fC), and 5-carboxycytosine (5caC) in early embryogenesis. Alice Chen (Stemgent Inc., USA) reported the generation of iPSCs via the efficient delivery of mRNA. Hitoshi Niwa (RIKEN, Japan; Nat. Cell Biol. 13, 1024-1026, 2011) reported recent progress regarding the network of transcription factors of Sox2 and Klf4. Sox2 transmits the signal to Tcfap2c in the development TS and Klf4 transmits the signal to Oct4 in the development of ESCs.

Differentiation mechanisms

Rong Lu and Irving Weismann (Stanford University, USA; Nat. Biotechnol. 29, 928-933, 2011) described the viral genetic barcoding with high-throughput sequencing to track the progeny of hematopoietic single cells after transplantation. Jennifer Antonchuk (TEMCELL Technologies Inc., Canada) reported the role of the Jmj-C-domain-containing histone demethylase Jhdm1a/Jhdm1b in nuclear reprogramming using vitamin C and miR302/367. Eric Deneault (University of Montreal, Canada) identified the 18 factors that enhance HSC activity and niche activity. This author and co-workers found that Sfpi1 enhanced LIF mRNA production. In osteoclastogenesis, Sfpi1 and Klf10 increased Tcfec and, in osteoclast-like cells, Prdm17 induced Sfpi1 and then Tcfec with the help of Klf10. Hongkui Deng (Peking University, China) reported the stepwise induction of hESCs or iPSCs to insulin-producing cells in a chemically-defined culture system. Most of these insulin-producing cells coexpressed beta cell-specific markers such as NKX6-1 and PDX1. The author and co-workers also reported the signaling pathways that regulate the maturation of pancreatic progenitor cells (CD24-Pdx1/Sox9/HNF6/HNF1b/NKK1) into functional beta cells and their application to STZ-induced diabetic mice or monkeys. Deepak Srivastava (University of California, San Francisco, USA; Cell 142, 375-386, 2010; Nat. Cell Biol., 13, 1244-1251, 2011) described the direct differentiation of disease-specific human iPSCs using a combination of human heart cardiac regulatory factors (GATA4/MEF2C/TBX5). These induced cardiomyocytes were shown to differentiate via *in vivo* reprogramming. Lijian Hui (Shanghai Institutes for Biological Sciences, China; Nature 475, 386-399, 2011) reported that mouse mesenchymal fibroblasts were induced directly to functional hepatocyte-like (iHep) cells via transduction of GATA4, HNF1α, and FOXa3 and inactivation of $p19^{Arf}$, and that transplanted iHep cells repopulated the livers of fumarylacetoacetate-hydrolase-deficient Fah(-/-) mice and rescued almost half of the recipients from death by restoring liver functions. Fiona M. Watt (Cambridge University, UK; Nat. Methods, 8, 915-916, 2011; Cell 144, 577-589, 2011; Cell 148, 33-45, 2012) reported the importance of niches to control self-renewal and differentiation using Type I collagen-coated adhesion islands (polyacrylamide hydrogels, Sulfo-SANPAH cross-linkers) and prepared human artificial micro-epidermis (composed of 5—10 cells) that captured the single stem cell or supported the self-renewal of epidermal stem cells. Feng Ma (Institute of Blood Transfusion, CAMS, China) established an efficient blood-cell-inducing system via coculture of hESCs/hiPSCs with murine AGM and fetal liver-derived stromal cells. hESC and hiPSC colonies grew and differentiated into mesoderm-like cells, and then to hematopoietic progenitor cells on Days 10 to 14. These cells were further induced to erythrocytes, mast cells (MCs), and eosinophils, mimicking the normal development of human erythrocytes. This author and co-workers also reported the efficient large-scale production of erythrocytes, MCs, and eosinophils derived from hESCs and hiPSCs.

Cellular interactions

Linheng Li (University of Kansas Medical Center, USA; Genes Dev. 25, 1928-1942, 2011) demonstrated that the H19 differential methylated domain (H19-DMD) region influences hematopoietic stem cell (HSC) fate and function. Hiromitsu Nakauchi (Institute of Medical Science, University of Tokyo, Japan; Cell 142, 787-799, 2010; Cell 147, 1146-1158, 2011) reported the generation of functional organs using $Pdx1^{-/-}$ blastocysts and a blastocyst complementation technique. This researcher injected wild-type rat iPSCs into mouse $Pdx1^{-/-}$ blastocysts, which resulted in the competent replacement of defective cells and the formation of pancreas almost entirely from the injected rat iPSCs-derived cells. Chimeric mice of $Pdx1^{-/-}$ genotype survived to adulthood without any signs of diabetes. This new technology seems to be useful for organ supply. Toshio Suda (Keio University, Japan; Cell Stem Cell 9, 247-261, 2011; Cell Stem Cell 9, 298-310, 2011; Cell Stem Cell 7, 391-402, 2010) described the glycolytic metabolism of HSCs. Normal HSCs maintain intracellular hypoxia, stabilize HIF-1α, and generate ATP via anaerobic metabolism. Guoping Fan (University of California, Los Angeles, USA; Cell Stem Cell 8, 604-605, 2011) reported DNA methylation in mouse ESCs. This author and co-workers used triple knockout (TKO) mice for DNMT1, DNMT3a, and DNMT3b to analyze the epigenomics of about 3,000 genes. 5mC was reduced to 5%, 5hmC was reduced to 0.5%, CpG became $<$10%, cell differentiation and development upregulated, and cell metabolism was downregulated. Several repressed genes were associated with H3K4me3, H3K27Kme3, and the PRC2 complex. The repressed genes were not correlated with loss of DNA methylation; rather, they were related to changes in the c-Myc and Jak/Stat pathways. More than 50% of the microRNAs were also deregulated in TKO mice. Hideyuki Okano (Keio University School of Medicine, Japan; Proc. Natl. Acad. Sci. USA, 108, 16825-16830, 2011) reported the transplantation of human iPSC-derived neural progenitor cells into the spinal cord nine days after injury. These cells differentiated into three neural lineages, without development of teratoma or tumors. They also induced remyelination and axonal regrowth of host $5HT^{+}$serotonergic fibers and participated in synaptogenesis with host neurons, promoting the recovery of locomotive function.

Molecular control

Joanna Wysocka (Stanford University of School of Medicine, USA; Nature 470, 279-283, 2010) reported enhancer elements that induced the formation of cranial neurons from hNCC (neural crest), among $>$3400 enhancer elements. This author

identified the role of NR2F1 and NRSF2 in craniofacial development. Lin Hu (University of California, Berkeley, USA; Nat. Cell Biol. 13, 1353-1360, 2011) demonstrated that miR-34 was a novel p53 target that played an essential role in somatic reprogramming. Both the Nanog and Sox2 genes were also putative targets of miR-34. Andrew Xiao (Yale School of Medicine, USA) reported the role of H2A.X in replication burden and showed that aberrant H2A.X deposition led to the silencing of the Chr 12qF1 locus. V. Narry Kim (Seoul National University, South Korea; Mol Cell 43, 1005-1015, 2011; Nature 475, 201-205, 2011) showed the presence of double-negative feedback of Lin 28 and Let7 between ESCs/cancer cells and differentiated cells. The Let 7 family of proteins is suppressed by the Lin 28 proteins (via recruitment of the terminal uridyltransferases TUT4 and TUT7) that bind to and induce the modification of pre-Let 7. U-tail of Uri-pre-Let 7a-1 inhibited Let 7 processing by suppressing Dicer function. Yukiko Gotoh (University of Tokyo, Japan; Nature Rev. Neurosci. 11, 377-388, 2011) reported the quiescence of neural stem cells (NSCs), as well as the molecular studies of niche signals and niche support of undifferentiated NSCs. The signal of p57 and the asymmetric distribution of delta-like 1 contributed to this event. Gang Wang (Shanghai Institute of Biochemistry and Cell Biology, CAS, China; Mol. Cell 45, 459-469, 2012) reported the role of the mediator Med23 in embryonic stem cell stemness. The deficiency of Med23 in ESCs promoted neural differentiation via the Erk signal, BMP, and Wnt. Shengxi Guan (New England Biolabs., USA) reported the new modification-specific restriction enzyme PvuRts1l, which recognizes 5hmC and 5-glucosylhydroxymethylcytosine (5ghmC) in double-stranded DNA and can be used for mapping genomic 5hmC. Zhaoyu Lin (Nanjing University, China) developed a two-phase analysis to induce iPSCs. Sox repressed TGFβ signaling, which is required for iPSC production. Haifan Lin (Yale University of School of Medicine, USA; Ann. Rev. Genet, 45, 447-469, 2011, Nat. Genet. 43, 153-158, 2010) reported the role of the YB body in piRNA biogenesis and as a gateway for Piwi expression and transport to the nucleus. Kat Hadjantonakis (Sloan-Kettering Institute, USA) reported the role of Sox17 in the formation of the ECM. Ling-Ling Chen (Shanghai Institute of Biochemistry and Cell Biology, CAS, China; Mol. Cell 35, 467-478, 2009) reported the role of long noncoding RNAs in human ESCs. These RNAs are uncapped and nonadenylated and appear to depend on snoRNA at both ends for proper processing; in addition, they are enriched in ESCs and iPSCs. Ricardo A. Rossello (Duke University, USA) demonstrated the conservative reprogramming of cells in all model organisms. Sheng Ding (UCSF, USA; Cell Stem Cell 9, 113-118, 2011) reported the direct reprogramming of human adult dermal fibroblasts (mesoderm) to functional neurons (ectoderm) using a combination of a microRNA (miR-124) and two transcription factors (MYT1L and BRN2) under precisely defined conditions. These human-induced neurons (hiNs) exhibit typical neuronal morphology and marker gene expression, fire action potentials, and produce functional synapses among each other.

Cell therapies

Lorenz Studer (Memorial Sloan-Kettering Cancer Center, USA; Nature 480, 547-551, 2011; Cell 145, 827-830, 2011; Cell Stem Cell, 8, 695-706, 2011; Nat. Biotechnol. 29, 233-235, 2011) discussed the efficacy of iPSCs as a treatment for familial dysautonomia (FD, Riley-Day syndrome, hereditary sensory and autonomic neuropathy type III), which is a rare genetic disorder of the peripheral nervous system. This author and co-workers demonstrated three disease-specific phenotypes in FD-iPS-derived cells that were partially rescued by treating cells with the plant hormone kinetin. They also reported the directed differentiation of pluripotent stem cells (PSCs) into specialized cells, such as spinal motor neurons or midbrain dopamine (DA) neurons. Kevin A D'Amour (ViaCyte Inc., USA) reported an encapsulated cell therapy for the insulin-dependent diabetes. This author and co-workers produced pancreatic progenitors *in vitro* (Pro-islets), followed by transplantation to sites where they act as beta cell precursors, at the GMP level and in a large scale. This encapsulation device enabled the survival of implanted Pro-islets, as well as their differentiation into functioning islet cells. Tariq Enver (University of Oxford, UK) reported the genome-wide identification of the targets of GATA-2 (stem/progenitor), GATA-1 (erythroid), and PU.1 (myeloid) at the single-cell level.

At the closing keynote address, Gordon Keller (University Health Network, Canada; Nat. Biotechnol. 29, 1011-1018, 2011; Cell Stem Cell 8, 228-240, 2011) reported the optimized lineage-specific differentiation of human pluripotent stem cells using the various signaling pathways of activin/nodal, Wnt, BMP, FGF, and VEGF/KDR and defined the signaling required for self-renewing and differentiation.

Conclusion

This meeting gave the impression that the field of stem cell research is maturing very fast, with an increase in the rate of the development of clinical applications for novel stem cell treatments. However, for this purpose, we need additional basic knowledge on the molecular mechanisms underlying cellular reprogramming. We feel that no researcher analyzed the quantitative level of the interaction between each signal and transcription factor and expected more from this interesting conference. Without quantification, it is very difficult to conclude on the expression of transcription factors in each signaling cascade. We still have not found the real defined factors or networks to determine 'stemness' in stem cells.

Shang-En Huang
Hsiang-Jung Hsiao
Chia-Chen Ku
Kazunari K. Yokoyama*
Graduated Institute of Medicine,
Kaohsiung Medical University,
Kaohsiung, Taiwan

*Corresponding author.
Graduated Institute of Medicine,
Kaohsiung Medical University,
100 Shih-Chuan 1st Road,
San Ming District, 807 Kaohsiung, Taiwan.
E-mail address: kazu@kmu.edu.tw (K.K. Yokoyama)